

Semester-Long Assessment of Aseptic Technique in Microbiology Labs

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INTRODUCTION

Biology educators emphasize aseptic technique when teaching lab courses. Aseptic technique is very important in microbiology to ensure safety and prevent cross-contamination. Although it can be assessed throughout the semester by examining the students' technique and observation of contamination, this tool offers a simple semester-long graded assessment of each student's technique. The procedures allow the students to perform a quick exercise that can determine if cross-contamination occurs, thus determining if aseptic technique was observed. Instructors can quickly check for the presence of cross-contamination. After implementing this procedure, a quantifiable improvement in my students' aseptic technique throughout the semester was observed. It was noted that between the middle and end of the semester there was a slight increase in the amount of cross-contamination but still well below the contamination observed at the beginning of the semester. This procedure was a valuable way to assess aseptic technique during the course of a semester.

A search for "aseptic technique" on the American Society of Microbiology website reveals that several lab experiments and job opportunities mention the importance of aseptic technique. It is a skill that must be used for all laboratory procedures when working with microorganisms. It is important for both the safety of the students and for the proper handling of microorganisms. There are several good explanations of aseptic technique on the Internet, and the Nuffield Foundation provides a very good description of techniques (1). The goal of this project is to provide instructors with a tool to assess the aseptic technique of their students by using simple transfers of bacteria to fresh media to identify cross-contamination.

When class experiments do not work students may not understand how poor aseptic technique impacted the project. This tool would work best for undergraduate students in an introductory microbiology course, so stu-

dents can see the effects of poor aseptic technique. Adding a graded assignment to their aseptic technique ensures their full effort in this key aspect of microbiology. The procedure can be presented during the first laboratory meeting as a way to explain the importance of aseptic technique and transferring cultures. It works well as an introductory lab since it also introduces the students to microorganisms and the need to incubate samples to observe growth. This semester I used this tool as a way to monitor the students' progress from the beginning to the end of the semester. I made it a part of their first class, the midterm practical, and their final practical.

PROCEDURE

Preparation of supplies

Liquid bacterial suspensions of *Serratia marcescens* and *Escherichia coli* are to be prepared for each student in the class. These are relatively safe strains that can be used in any lab. Only pigmented *Serratia marcescens* (Carolina Biological Supply, #155433) can be used for the procedure. To prepare the liquid suspensions, remove an isolated colony of *S. marcescens* from a plate and transfer it to either tryptic soy broth (TSB) or Luria broth (LB) and repeat the same procedure with *E. coli*. Incubate both broths at 37°C so the *S. marcescens* does not produce its pigment.

Student activity

Each student will be provided with one suspension of *S. marcescens* and one suspension of *E. coli*, two tubes of either LB or TSB, and one metal inoculating loop (no disposable inoculating loops). Have each student use the following procedure:

1. Transfer a loopful of the *S. marcescens* suspension to a sterile tube of LB or TSB using the sterile inoculating loop. It is best to have the students transfer the *S. marcescens* to the sterile broth tube before transferring the *E. coli* to make grading easier for the instructor.
2. Repeat the procedure for the *E. coli* suspension.
3. Incubate the tubes at 25°C for 48 hours.

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Assessment

To assess the students' aseptic technique, the instructor will need to observe the prepared tubes at 48 hours after the inoculation. If red pigmentation is present in the *E. coli* tube, then there is cross-contamination from *S. marcescens*. To confirm this assessment a three-phase streak of each sample can be performed on tryptic soy agar (TSA). The plates are then incubated at 25°C for 48 hours. With the three-phase streak, *E. coli* plates that are cross-contaminated will show red pigmentation, usually in the primary streak. As a way to allow for more learning opportunities for the class, each student can perform the three-phase streak on their own samples after the incubation. This step can be used to further evaluate the students' aseptic technique. The presence of airborne contaminants on the surface of the TSA plate will show if the students are using proper aseptic technique when working with solid media. This step also provides additional practice for the students in performing a three-phase streak and being able to isolate colonies.

CONCLUSION

Adding a graded assignment that focuses on aseptic technique helps place emphasis on this important component of a microbiology lab. During the semester, this procedure was used in two lab sections (46 students), and the students' aseptic technique was periodically assessed. The exercise was performed during the first lab period, after an explanation of what needed to be completed to ensure there was no cross-contamination. The students also performed the procedure during the midterm practical and the final practical of the semester. Figure 1 shows the percentage of observed cross-contamination on the initial assessment, the midterm practical, and the final practical of the semester.

The class data showed an improvement in aseptic technique from the beginning of the semester to the end of the semester. The class average on the midterm was better than on the final lab practical. However, there can be several reasons for the increase in cross-contamination on the final exam. The increase in cross-contamination from the midterm to the final showed that aseptic technique needs to be emphasized throughout the semester since some students can become "sloppy" with the technique when they are in a busy semester.

Each week in microbiology lab, students perform different techniques and work on different projects. Aseptic technique will always be mentioned but might not be

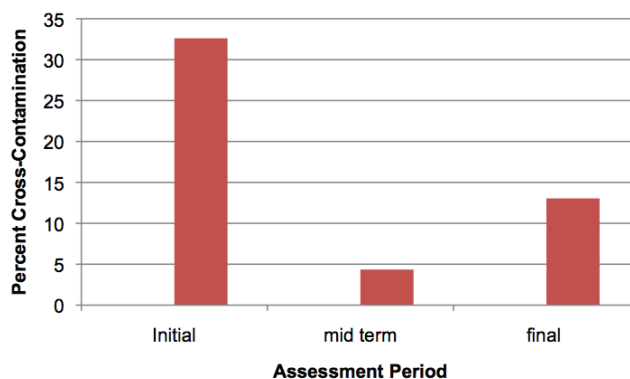


FIGURE 1. Assessment of aseptic technique results for two microbiology classes using the tube transfers. The test was performed on the first day of class, during the midterm exam, and during the final exam. The percentage of observed cross-contaminated tubes is shown.

emphasized as part of the new procedures. Students need a way to constantly have aseptic technique reinforced. This exercise can be used to keep the students focusing on aseptic technique throughout the semester without putting much additional work on the instructor. This procedure allows the instructor an objective way to analyze the students' aseptic technique. By providing a grade for each student's technique, the importance of aseptic technique is reinforced. While the preparation of the supplies can become time-consuming, depending on the size of the class, the assessment of the results is not very demanding on the instructor.

ACKNOWLEDGMENTS

The author declares that there are no conflicts of interest. The project was conducted using students in a classroom setting. There was IRB approval for the research. The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories, available at www.asm.org.

REFERENCES

1. Nuffield Foundation. 2009. Aseptic techniques. <http://www.nuffieldfoundation.org/practical-biology/aseptic-techniques>. Accessed November 24, 2009.